

ENDOGENOUS FECAL LOSSES OF CALCIUM COMPROMISE CALCIUM BALANCE IN PANCREATIC-INSUFFICIENT GIRLS WITH CYSTIC FIBROSIS

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Objective Bone mineral density is compromised in individuals with cystic fibrosis (CF); calcium is the major bone mineral. This study examined the impact of endogenous fecal calcium (V_{endo}) losses on calcium balance in girls with CF.

Study design V_{endo} was measured in 12 girls with CF (aged 7-18 years): 7 younger, premenarcheal girls with compromised nutritional status; and 5 older, postmenarcheal girls with adequate nutritional status. V_{endo} was measured as the amount of intravenously administered ^{42}Ca , a calcium stable isotope, in stool relative to urine over 6 days. V_{endo} was compared between pre- and postmenarcheal girls by Student's t test. Actual calcium balance [absorbed calcium – (urinary calcium (V_{u}) + V_{endo})] was compared with estimated balance (assuming $V_{\text{endo}} = 1.6 \text{ mg/kg/day}$ calcium) by paired t test.

Results V_{endo} was $99.3 \pm 42.3 \text{ mg/day}$. By body weight, V_{endo} was highest among premenarcheal girls ($3.37 \pm 1.09 \text{ mg/kg/day}$), resulting in excess losses ($>1.6 \text{ mg/kg/day}$) of $55.0 \pm 45.7 \text{ mg/day}$. Over 1 year, this represents $20.1 \pm 16.7 \text{ g}$ of unattained bone calcium or $6.7 \pm 4.2\%$ of the bone calcium content of these girls.

Conclusions V_{endo} is a significant source of calcium loss in individuals with CF and may limit calcium availability for bone mineral deposition. (*J Pediatr* 2003;143:765-71)

Although pulmonary failure accounts for more than 80% of deaths in cystic fibrosis (CF), the function of the gastrointestinal tract also is adversely affected, resulting in pancreatic insufficiency, maldigestion, increased intestinal permeability, and, ultimately, in compromised nutritional status.^{1,2} As the life span of persons with CF increases, reduced bone mass leading to osteoporosis has emerged as an important secondary consequence of the disorder.^{3,4} Although the etiology of reduced bone mass in patients with CF is likely to be multifactorial, speculation has existed that altered calcium metabolism—in particular, compromised calcium absorption—may be an important contributor.^{5,6} Calcium is the major mineral of bone, comprising 32.2% of bone mineral content,⁷ and, as such, alterations in the ability to acquire or retain calcium would limit the amount of substrate available for bone mineralization.

Little data actually exist examining components of calcium balance in persons with CF. Using a dual isotope approach, we reported recently that calcium absorption and urinary calcium excretion (V_{u}) were similar in girls with CF, nearly 50% of whom had evidence of reduced bone mass (lumbar spine bone mineral density [LS BMD] Z score <-1), compared with healthy girls.⁸ Because these aspects of calcium balance were normal in the girls with CF, we hypothesized that excessive loss of endogenous fecal calcium (V_{endo}) may have been present, thereby limiting calcium retention.

V_{endo} comprises calcium of nondietary sources that is secreted into the gastrointestinal tract during the process of digestion and is lost in the feces. Typically, in healthy persons approximately one third of the calcium secreted into the intestinal lumen is reabsorbed, whereas approximately two thirds of this calcium is lost in the feces. These losses are derived

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CF	Cystic fibrosis	TBBMC	Total body bone mineral content
BMI	Body mass index	V_{endo}	Endogenous fecal calcium
FEV ₁	Forced expiratory volume at one second	V_{u}	24-hour urinary calcium excretion
LS BMD	Lumbar spine bone mineral density		

from pancreatic or bile secretions, secretion of serosal calcium across the epithelium into the intestinal lumen, and the sloughing of cells of the digestive tract. In more than 500 studies of healthy adult women, these losses averaged 102 mg/day (1.6 mg/kg/day), were positively associated with body size, and were negatively associated with fractional calcium absorption.⁹ Furthermore, in these women, V_{endo} was only minimally affected by calcium intake.¹⁰ Two studies have characterized V_{endo} in a total of 30 healthy children (3–14 years of age) using stable isotopes of calcium. Data from these children demonstrated that V_{endo} averaged 1.6 mg/kg of body weight/day and is unrelated to age, sex, pubertal status, or calcium intake across the range of 1180 to 1480 mg/day.^{11,12}

Because of the many digestive and gastrointestinal difficulties present in CF, V_{endo} losses may be elevated as a consequence of CF, as has been found for zinc.¹³ High V_{endo} could be attributable to excessive calcium secretion into the intestinal lumen, its compromised reabsorption, or both. It is particularly important to examine this aspect of calcium balance during puberty in persons with CF, as it is at this time of life when peak rates of bone calcium acquisition are achieved.¹⁴ The purpose of this study, therefore, was to describe V_{endo} and its impact on calcium balance in a group of clinically stable, pancreatic insufficient girls with CF.

SUBJECTS AND METHODS

Protocol

As part of a larger calcium kinetic study, girls from 7 to 18 years of age were recruited from The Johns Hopkins Hospital Cystic Fibrosis Center and from two other regional CF centers (Harrisburg, Penn, and Wilmington, Del). The study was approved by The Johns Hopkins Joint Committee on Clinical Investigation, and assent and consent were obtained from each subject and her parent or guardian. Details of the larger study of calcium absorption and (V_u) in 23 girls have been reported previously.⁸

Data on V_{endo} were derived from 12 pancreatic insufficient participants (from the larger cohort of 23 girls) who were compliant with a 6-day stool collection, as determined by meeting at least two of three criteria: (1) collection of at least one stool sample per day; (2) at least 100 g/day of stool provided; (3) a recovery of calcium in stool >50%, where total stool calcium recovery equaled calcium intake (4 day average) minus total absorbed calcium (fractional absorption \times calcium intake). This represents a rough minimum estimate of the calcium that would be expected to remain in stool after dietary calcium is absorbed, without accounting for stool calcium contributed by V_{endo} . Data from one pancreatic sufficient study subject and from 10 subjects in whom stool sample collections were assumed to be incomplete because they did not meet the described criteria were excluded from analysis.

Subjects were clinically stable (absent of pulmonary exacerbations) and generally compliant with their CF treatment regimens, as reported by the clinical staff treating their CF. They had not taken oral glucocorticoid preparations for at least one month before participating in the study. All

prescribed medications were continued during the course of the study, including pancreatic enzymes.

Fasted girls were admitted in the morning before breakfast for a 24-hour stay to The Johns Hopkins Pediatric Clinical Research Unit. A fasting blood sample (15 mL) was obtained, and girls were given ⁴⁴Ca (0.35 mg/kg of body weight) in whole milk with breakfast. After breakfast, ⁴²Ca (0.2 mg/kg of body weight) was administered intravenously through a heparin lock. Complete 24-hour urine and stool collections were obtained after tracer administration. Girls self-selected their foods during the 24-hour stay, and foods were pre- and postweighed to obtain exact intakes.

While the subjects were in the research unit, their weight and height were recorded, and these data were compared with the current growth references to obtain age appropriate Z scores for weight, height, and body mass index (BMI).¹⁵ Tanner growth stage of pubertal development (based on breast development) was assessed by a pediatric endocrinologist.

Total body bone mineral content (TBBMC) and body composition were obtained for each girl by dual-energy x-ray absorptiometry using a Hologic QDR-4500A (Hologic, Inc, Bedford, Mass), and LS BMD Z scores were obtained. TBBMC was compared with that expected, based on a prediction equation developed from data of 483 healthy girls that accounts for age, height, and race,¹⁶ thereby providing an appropriate reference for girls with CF, who are expected to be small compared with healthy girls of the same age. Actual TBBMC of the girls with CF was expressed as a percentage of that predicted by this equation. Estimates of total body calcium content were made by multiplying TBBMC by 32.2%; conversely, estimates of the contribution of calcium retention obtained by calcium balance to TBBMC were made by dividing calcium retention (converted to g/year) by 32.2%, the fraction of bone mineral content made up of calcium.⁷

To encourage participation, girls were allowed to return home after the 24-hour inpatient portion of the study to collect three spot urine samples daily and to complete stool output in calcium-free containers for the subsequent 5 days. Thus, a total of 6 days of stool output was obtained post-dosing, including the first inpatient day.

After returning home, subjects also were asked to carefully record their dietary intake for 3 days so that information on typical dietary calcium intake could be ascertained. Diet records were analyzed for nutrient intake using the Minnesota Nutrition Data System (Version 2.91, University of Minneapolis, Minn). Estimates of daily calcium and energy intake were derived from the average of the 4 days of data collection (1 inpatient day and 3 days of home records).

A clinical status score was derived from forced expiratory volume in one second (FEV₁) measures obtained at a regular clinic visit within 2 months of the time of the study. Severity of lung disease was characterized according to FEV₁ score as follows: normal ($\geq 90\%$ of expected); mild (70%–89% of expected); moderate (40%–69% of expected); or severe (<40% of expected). Estimates of daily pancreatic enzyme intake were standardized based on the prescribed enzyme dose assuming three meals and two snacks were eaten daily.

Laboratory Analysis

Calcium content of each 24-hour urine collection and of each spot urine sample was measured using atomic absorption spectrophotometry (Perkin Elmer model 3300, Norwalk, Conn).

Stool samples were weighed and homogenized in blenders with a measured amount of deionized water. A 5- to 10-g sample of the homogenate was dried to a constant weight to determine dry weight. Total water content was calculated as the difference between the wet and dry weight, and the proportion of added water was accounted for in the calculations. Dried stool samples were dry-ashed in a muffle furnace at 500°C, then further digested with repeated additions of Ultrex nitric acid (JT Baker, Phillipsburg, NJ) on a hot plate. Digests were evaporated to dryness, dissolved to completion in acid, and diluted to 100 mL in deionized water. Total calcium was measured in each diluted solution by atomic absorption spectrophotometry (Perkin Elmer model 3300). The calcium content of each stool sample was then calculated after accounting for the dilution factor and added water.

Fecal fat was measured at the United States Department of Agriculture Beltsville Human Nutrition Research Center in Maryland. Aliquots of blended stool samples were freeze-dried, and the fat analysis was performed in duplicate with a CEM Corporation FES 80 fat extractor (Matthews, NC) using methylene chloride as the extraction solvent. Fecal fat was expressed as a fraction of fat intake based on the average daily intake of fat determined from dietary records.

Isotope Analysis and Calculations of Endogenous Fecal Calcium

Calcium was extracted from urine samples and stool sample solutions by precipitating with ammonium oxalate.¹⁷ For urine samples, extracted calcium was loaded onto a rhenium filament, and the $^{42/48}\text{Ca}$ isotopic ratio was measured using a quadrupole thermal ionization mass spectrometer (Finnigan THQ, Bremen, Germany) at The Johns Hopkins University. Enrichments obtained with this instrument were cross-validated with those obtained at the Baylor School of Medicine. Because of the lower enrichment of intravenous tracer in stool samples, the $^{42/43}\text{Ca}$ isotope ratio was measured using a more precise magnetic sector thermal ionization mass spectrometer (Finnigan 261) at the Baylor School of Medicine. Ratios were corrected for isotopic fractionation, and the enrichment of tracer was expressed as the Δ percent excess, the degree to which the measured ratio was increased over the natural abundance ratio.

V_{endo} was calculated as V_u (mg/kg/day) multiplied by the ratio of the cumulative appearance of ^{42}Ca in stool relative to the cumulative appearance in urine over the 6-day study period, according to the following equation:

$$V_{\text{endo}} = V_u \times \left(\int ^{42}\text{Ca in stool} / \int ^{42}\text{Ca in urine} \right)$$

Cumulative appearance of ^{42}Ca in urine and stool was obtained from the following equation, where 0.647% re-

presents the naturally occurring percentage of calcium that exists as ^{42}Ca .

Cumulative $^{42}\text{Ca} = \sum (\% \text{ excess } ^{42}\text{Ca} \times \text{total calcium output} \times 0.647\%) / \text{total } ^{42}\text{Ca dose}$ For urine, cumulative ^{42}Ca appearance was obtained by adding ^{42}Ca outputs for each day. The average excess of ^{42}Ca from the three spot urine samples and the calcium content of the original 24-hour urine collection were used in the equation to determine cumulative ^{42}Ca appearance for the 6-day period. For stool, ^{42}Ca excess and total calcium output were obtained for each pooled sample of stool.

The methodology for this study was similar to that described by Abrams et al in a study of V_{endo} using stable calcium isotopes in children.¹¹ That study showed that in healthy children, recovery of tracer excess in urine was nearly complete (>99%) by 5 days postdosing, whereas in at least one child, a 10-day collection was required for stool tracer excess to reach asymptotic values. Because a sample collection period longer than 6 days was thought to be unreasonable for our study subjects, excretion of tracer into stool may have been incomplete in this study, leading to an underestimate of the cumulative appearance of ^{42}Ca in stool and, thus, to an underestimate of actual total V_{endo} .

Data Analysis

Results are expressed as the mean \pm SD. Because our sample of girls fell into two distinct categories, data are presented separately for premenarcheal ($n = 7$; 5 girls were Tanner stage 1, and 2 girls were Tanner stage 2) and postmenarcheal ($n = 5$, all were Tanner stage 5) groups, and comparisons between groups were assessed with Student's t test. Nutritional status was compared with data compiled nationally by the Cystic Fibrosis Foundation¹⁸ to determine how comparable our sample was with the national average.

V_{endo} was compared by Student's t test with values reported in 9- to 14-year-old children obtained using similar methodology.¹² An estimate of the impact of V_{endo} on calcium balance was made by comparing the actual mass balance [total calcium absorbed $-(V_u + V_{\text{endo}})$] with the balance calculated assuming $V_{\text{endo}} = 1.6$ mg/kg/day, as reported in healthy children.¹² The difference between estimated and actual balance within each study subject was tested using a paired t test. We looked for associations of V_{endo} with a variety of variables using linear regression. All data were analyzed using STATA v. 7.0 (StataCorp, College Station, Tex), and differences were considered significant at $P < .05$.

RESULTS

Among our sample, 5 girls (42%) previously had suffered at least one fracture. All but 1 of the girls reported being at least moderately active. Five girls (42%) were homozygous for the ΔF508 mutation, 3 (25%) were heterozygous, 1 had other unidentified mutations, and data for 3 girls were unavailable. Six of the 7 premenarcheal girls (86%) and 2 of the 5 postmenarcheal girls (40%) were using inhaled steroid

Table I. Subject characteristics

	Premenarcheal (n = 7)		Postmenarcheal (n = 5)	
	Mean \pm SD	Range	Mean \pm SD	Range
Age (y)*	9.7 \pm 1.6	7.6-11.5	16.1 \pm 1.7	14.3-17.9
Weight (kg)*	29.6 \pm 8.0	20.4-45.2	57.8 \pm 10.8	44.8-73.2
Weight Z score	-0.62 \pm 0.73	-1.66 to 0.70	0.18 \pm 1.21	-1.72 to 1.50
Height (cm)*	133.8 \pm 9.8	121.0-149.2	162.7 \pm 7.05	155.0-170.6
Height Z score	-0.50 \pm 0.76	-1.85 to 0.35	0.08 \pm 1.08	-1.25 to 1.32
BMI (kg/m ²)*	16.2 \pm 2.1	13.9-20.3	21.7 \pm 2.4	18.6-25.2
BMI Z score	-0.39 \pm 0.66	-1.22 to 0.83	0.25 \pm 0.85	-1.01 to 1.21
LS BMD Z score	-0.92 \pm 0.66	-1.77 to 0	0.13 \pm 1.54	-2.52 to 1.45
TBBMC (g)*	915 \pm 278	612-1448	2011 \pm 351	1518-2328
TBBMC% (% of predicted)	91.2 \pm 10.3	77.5-108.9	104.5 \pm 12.8	83.2-117.9
FEV ₁ (% of expected) [†]	97.5 \pm 9.1	81-104	58.5 \pm 15.7	35-68
Stool fat (% of intake)	8.7 \pm 5.8	2.3-16.3	17.4 \pm 14.7	0.9-37.9
Enzyme dose* (10 ³ IU lipase/kg/d)	8.6 \pm 1.6	6.3-10.3	5.9 \pm 1.7	3.5-8.0
Parathyroid hormone (pg/mL)	27.7 \pm 14.2	12.4-49.7	27.9 \pm 14.1	9.6-47.9

BMC%, percent of predicted bone mineral content for age and height.

*Significant difference between pre- and postmenarcheal girls, $P = .0001$.

[†]Significant difference between pre- and postmenarcheal girls, $P < .05$.

preparations at the time of the study. Six of the 7 premenarcheal girls (86%) were taking both acid-inhibitors and antibiotics; 2 of the 5 postmenarcheal girls (40%) were taking these preparations. One of the postmenarcheal girls had CF-related diabetes mellitus, and one used a gastrostomy tube for enteral feeds. Two of the postmenarcheal girls reported missing the collection of one stool sample during the 6-day period.

Postmenarcheal girls were older, heavier, and taller than the premenarcheal girls (Table I). BBMC was double that of the premenarcheal girls, and their average LS BMD Z scores were ~ 1 SD higher. However, because of small sample numbers and variability in these measures, weight, height, BMI, and LS BMD Z scores did not statistically differ between the premenarcheal and postmenarcheal girls. By LS BMD Z scores, 4 of the premenarcheal girls (57%) were osteopenic (LS BMD Z score from 1 to 2 units below zero), and the remaining 3 (43%) had LS BMD Z scores at or below zero. One of the postmenarcheal girls (20%) was osteoporotic (LS BMD Z < -2); the others (80%) had positive LS BMD Z scores. By examining the percent of actual to predicted TBBMC, 2 premenarcheal girls (29%) and 1 postmenarcheal girl (20%) were osteopenic (ranging from 76%-88% of that expected).

Average weight, height, and BMI Z scores were equivalent to the 27th, 31st, and 35th percentiles, respectively, for the premenarcheal girls. Corresponding percentile values were 57th, 53rd, and 60th, respectively, for the postmenarcheal girls. According to the Cystic Fibrosis Foundation data registry, girls with CF in this age range typically average between the 20th and 40th percentiles for these measures.¹⁸ Therefore, the nutritional status of the premenarcheal girls with CF was comparable to that reported nationally, whereas average nutritional status of the postmenarcheal

girls with CF was substantially better than that reported nationally.

FEV₁ was higher among the premenarcheal girls with CF, which would be predicted from reported age-related changes in lung function.¹⁸ Enzyme doses were higher among the premenarcheal girls with CF, and fecal fat, as a percentage of fat intake, was not significantly different between groups. Parathyroid hormone concentrations were similar between groups and were not elevated.

Mean V_{endo} , expressed in mg/day or as a proportion of body weight (mg/kg/day), is presented in Table II. There was no difference in total V_{endo} between pre- and postmenarcheal girls with CF. Average losses for both groups with CF, 99.3 \pm 42.3 mg/day, were significantly higher ($P = .01$) than those reported for 13 healthy girls (61.2 \pm 27.7 mg/day).¹² The premenarcheal girls with CF in our study were approximately 2 years younger and weighed 13 kg less than the healthy girls in that study.

When expressed relative to body weight, V_{endo} was significantly higher among premenarcheal girls with CF than among the postmenarcheal group. V_{endo} in the premenarcheal girls with CF was also substantially higher than that reported in 25 healthy children, in whom average V_{endo} was 1.6 \pm 0.8 mg/kg/day ($P < .0001$).¹² Among the postmenarcheal girls with CF, V_{endo} was higher in 2 girls who took acid inhibitors than in those who did not (2.0 \pm 0.2 vs 1.4 \pm 0.1 mg/kg/day, $P = .02$).

In Table III, estimates of calcium balance obtained by assuming $V_{\text{endo}} = 1.6$ mg/kg/day and those obtained by using actual measures of V_{endo} are compared. Average calcium intake and the amount of absorbed calcium were similar between girls with CF in both pubertal groups, and V_u was somewhat higher among the postmenarcheal girls with CF ($P = .053$). Among our sample of postmenarcheal girls, an

Table II. Endogenous fecal losses of calcium in girls with cystic fibrosis*

V_{endo}	Premenarcheal (n = 7)	Postmenarcheal (n = 5)
mg/d	102.4 ± 55.5	95.0 ± 16.1
mg/kg/d [†]	3.37 ± 1.09	1.68 ± 0.35

*Mean ± SD.

[†]Significant difference between pre- and postmenarcheal girls, $P < .01$.

estimate of 1.6 mg/kg/day would have adequately characterized calcium balance; however, among the premenarcheal girls, calcium balance would have been overestimated by an average of 55 mg/day using an estimate for V_{endo} of 1.6 mg/kg/day. Actual calcium retention was 73% ($\pm 17\%$) of that which would have been retained if rates of V_{endo} among the premenarcheal girls with CF were comparable to that of healthy children.

Extrapolating the difference in estimated and actual daily calcium retention across one year's time among the premenarcheal girls with CF, actual calcium balance would be 20.1 ± 16.7 g ($P = .02$) less than the potential for bone calcium acquisition if V_{endo} were 1.6 mg/kg/day; this difference is equivalent to $6.7 \pm 4.2\%$ of the current bone calcium content of these girls (significantly different than 0%, $P < .01$). An estimated daily calcium retention of 227 ± 154 mg/day extrapolated over one year's time would be equivalent to an increase in TBBMC of 257 ± 174 g/year, whereas the actual daily calcium retention of 172 ± 127 mg/day extrapolated over one year's time would be equivalent to an increase in TBBMC of only 195 ± 144 g/year.

Among girls in both pubertal groups, BMI Z score was positively associated with total V_{endo} such that larger girls, as expected, excreted more total calcium through this route (17.6 mg increase in V_{endo} for each unit increase in BMI Z score, $r = .6$, $P = .05$, excluding outlying data from one participant that exaggerated this relationship). No relationships were seen with calcium intake, absorption, pancreatic enzyme intakes, fecal fat excretion, or parathyroid hormone concentration. LS BMD and TBBMC% Z scores were not associated with V_{endo} .

DISCUSSION

We directly measured V_{endo} in children with CF. It is particularly important to consider the impact of these losses on calcium balance during the pubertal growth spurt, when the greatest opportunity exists for maximizing calcium retention in support of bone calcium accretion. Our findings indicate that V_{endo} losses were higher among girls with CF than among healthy children. V_{endo} losses significantly compromised calcium retention in the younger and smaller children, in whom V_{endo} represented a larger proportion of total body calcium content. The difference in V_{endo} relative to body weight between pre- and postmenarcheal girls with CF observed in this study is most likely explained by the heterogeneous expression of the disease between girls with CF in the two pubertal groups. The possibility that V_{endo}

Table III. Impact of endogenous fecal calcium losses on calcium balance*

	Premenarcheal (n = 7)	Postmenarcheal (n = 5)
Calcium intake (mg/d)	1420 ± 205	1350 ± 528
Actual calcium absorbed (mg/d)	355 ± 121	277 ± 110
V_u (mg/d)	80.8 ± 45.9	145.7 ± 56.9
Estimated calcium balance (mg/d) [§]	227 ± 154	39 ± 99
Actual calcium balance (mg/d) [‡]	172.0 ± 127.0	36.7 ± 97.4
Difference in balance (mg/d)	55.0 ± 45.7	2.6 ± 19.1

*Mean ± SD.

[†]Calculated as actual calcium absorption - ($V_u + V_{\text{endo}}$), where $V_{\text{endo}} = 1.6$ mg/kg/d.

[‡]Calculated as actual calcium absorption - ($V_u + V_{\text{endo}}$), where V_{endo} is measured directly.

[§]Significant difference between pre- and postmenarcheal girls, $P < .05$.

^{||}Within individual difference between actual and estimated calcium, balance is significantly different than zero, $P = .02$.

relative to body weight improves over the course of puberty in children with CF could only adequately be tested with a longitudinal study design, but differences in V_{endo} with pubertal stage have not been observed in healthy children.¹²

The premenarcheal girls with CF in this study had compromised nutritional status, consistent with a national CF database.¹⁸ TBBMC was approximately 9% lower than that expected based on their height and age,¹⁶ and average LS BMD Z scores were 1 unit lower than among the postmenarcheal group with CF. Increased use of pancreatic enzymes and acid inhibitors among the premenarcheal group further indicate that gut function may have been more substantially compromised among these girls. Although no direct association of LS BMD Z scores with V_{endo} losses was found among the younger girls, it was not surprising to find higher V_{endo} losses among them as a group. Thus, despite high levels of ingested and absorbed dietary calcium, the premenarcheal girls in this study are starting the time of maximal bone mineral accretion with compromised bone mineral status and excessive (>1.6 mg/kg/day) V_{endo} losses. Bailey et al determined based on serial dual-energy x-ray absorptiometry scans in healthy pubertal girls that peak rates of bone mineral acquisition averaged 322 g/year, equivalent to 284 mg/day of acquired calcium.¹⁴ Daily calcium retention and estimates of yearly increases in TBBMC among the premenarcheal girls with CF in this study approached the rates observed in healthy girls when $V_{\text{endo}} = 1.6$ mg/kg/day was assumed. However, when the contribution of measured V_{endo} losses were considered, daily calcium retention and estimated yearly increases in TBBMC fell considerably short of those observed among healthy girls.

In the postmenarcheal girls with CF in this study, average calcium balance was nearly identical to that reported

for healthy girls consuming approximately 400 mg less calcium daily¹⁹ despite their relatively high dietary calcium intake and low V_{endo} relative to body weight. Calcium balance in the postmenarcheal girls with CF was not different than zero, suggesting that significant gains in bone mass were no longer occurring in these girls.

The dual stable isotope methodology we utilized does not allow us to distinguish the source of the calcium secreted into the gastrointestinal tract or to distinguish whether V_{endo} is derived from excessive calcium secretion, impaired reabsorption of secreted calcium, or both. Calcium secreted into the gastrointestinal tract is thought to be subject to the same regulation of absorption as dietary calcium, although calcium secreted lower in the gastrointestinal tract would have less opportunity to be reabsorbed.⁹ Calcium secreted in excess from pancreatic and bile secretions released into the duodenum is an unlikely source of V_{endo} in our study subjects, because the calcium content of the duodenal juice of children with CF is reportedly lower than that of healthy children.²⁰ Because fractional absorption of calcium in these girls with CF was comparable to that observed in healthy children,⁸ it is unlikely that the capacity to reabsorb calcium secreted into the gastrointestinal tract would have been impaired in our subjects. Furthermore, the lack of relationship of V_{endo} with fecal fat suggests that coprecipitation of secreted calcium with luminal fats was not the primary cause of increased endogenous calcium losses in these children with CF, in contrast to a prevalent theory to explain calcium malabsorption in diseases where steatorrhea exists.²¹

We speculate that excessive V_{endo} in patients with CF is attributable to excessive secretion of calcium into the gastrointestinal tract. Interestingly, precedence exists for elevated V_{endo} losses but normal calcium absorption in other disease states associated with protein-losing enteropathies, including untreated celiac disease,²² protein-losing enteropathy associated with intestinal lymphangiectasia,²³ and among patients with Crohn's disease with protein-losing enteropathy.²⁴ This suggests that excessive serosal-to-luminal calcium secretion may contribute to elevated V_{endo} losses in diseases where tight junction integrity of the gastrointestinal tract is compromised.

Intestinal permeability is known to be 4 to 10 times greater in persons with CF than in healthy persons.²⁵ Increased permeability is thought to occur through compromised tight junctions of the intestine, allowing for passage of large, water-soluble molecules.²⁵⁻²⁷ Evidence of efflux of serum proteins such as albumin and IgG into the gastrointestinal tract of children with CF has been found,²⁸ and morphologic abnormalities have been demonstrated in the apical portion of goblet cells but not in cells responsible for nutrient absorption in the villi.²⁹ Calcium is secreted across the gastrointestinal tract in a paracellular manner through tight junctions, and serosal-to-luminal secretion may be greater than calcium absorption in the jejunum and ileum, based on in vitro studies in rat tissue.³⁰ All of these findings are consistent with the possibility of excessive serosal-to-luminal calcium transit in persons with CF.

Increased intestinal permeability in persons with CF may be a primary consequence of the genetic defect of CF, as the degree of intestinal permeability has been related to the $\Delta F508$ genotype,²⁷ or it may be related secondarily to pancreatic insufficiency.^{25,26} Intestinal permeability was partially corrected by year-long treatment with proton pump inhibitors in 14 children with CF, suggesting that luminal acid content directly or indirectly leads to damage of tight cell junctions.³¹ Acidity of the gastrointestinal tract leads to bile acid precipitation,³² and elevated fecal concentrations of bile acids have been shown in children with CF.³³⁻³⁵ Bile salts have been shown to increase colonic permeability of tight junctions in in vitro systems,³⁶ thereby suggesting a mechanism through which enhanced acidity may indirectly impair tight junction integrity of the gastrointestinal tract in CF. In this study, V_{endo} was high among premenarcheal girls, who were nearly universally taking gastric acid inhibitors, as well as in 2 postmenarcheal girls taking acid inhibitors.

To summarize, V_{endo} losses among clinically stable girls with CF were higher than those reported for healthy children. These calcium losses were especially high among girls with poorer nutritional and bone status, higher pancreatic enzyme intake, and higher use of gastric acid inhibitors. We speculate that increased secretion of serosal calcium occurs through permeable tight junctions in the gastrointestinal tract. This possibility represents a novel way of thinking about physiologic conditions in patients with CF that may contribute to compromised calcium balance and suggests that enhancing the availability of dietary calcium alone may be insufficient to optimize total body calcium retention. Further research into the magnitude, source, and control of V_{endo} losses among persons with CF is warranted. The potential impact of these additional losses of calcium on bone calcium deposition in children with CF additionally should be explored.

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